Appl. No. 10/580,544

Atty. Ref.: 3608-8

Amendment

April 5, 2011

**AMENDMENTS TO THE CLAIMS:** 

Please amend the claims as follows:

Claims 1-36. (Cancelled)

37. (Currently Amended) A method for preparing cross-linked polyelectrolyte

multilayers films, wherein said method comprises the reaction of complementary

functional groups: carboxylic groups and amino groups, present in the polymers that

constitute the multilayer film, in the presence of a coupling agent, as to form amide

bonds, wherein the reaction of carboxylic groups and amino groups of the

polyelectrolyte multilayers in the presence of a coupling agent is carried out also in the

presence of N-hydroxysuccinimide compounds,

wherein the multilayers comprise at least one layer pair of cationic

polyelectrolytes and anionic polyelectrolytes and the number of said layer pairs is from 5

to 60.

38. (Currently Amended) The method according to claim 37, wherein the used

polyelectrolyte multilayers are assembled via any complementary interaction, especially

electrostatic attraction and hydrogen bridging.

39. (Previously Presented) The method according to claim 37, wherein the

polyelectrolyte multilayers films are biocompatible.

Claim 40. (Canceled)

Claim 41. (Canceled)

42. (Previously Presented) The method according to claim 37, wherein said

carboxylic groups and amino groups are attached by covalent bonds to polyelectrolytes.

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43. (Previously Presented) The method according to claim 37, wherein the

polymers that constitute the multilayer film comprise cationic polyelectrolytes which

present free amino groups and anionic polyelectrolytes which present free carboxylic

groups.

44. (Currently Amended) The method according to claim 37, wherein the

polymers that constitute the multilayer film comprising anionic polyelectrolytes which

present free carboxylic groups are selected [[in]]from the group consisting of polyacrylic

acid, polymethacrylic acid, acid, poly(D,L-glutamic) acid, polyuronic acid (alginic,

galacturonic, glucuronic...), glycosaminoglycans (hyaluronic acid, dermatan sulphate,

chondroitin sulphate, heparin, heparan sulphate, and keratan sulphate), poly(D,L-

aspartic acid), any-combination of [[the]] polyamino acids, and mixtures thereof.

45. (Currently Amended) The method according to claim 37, wherein the

polymers that constitute the multilayer film comprising cationic polyelectrolytes which

present free amino groups are selected [[in]]from the group consisting of poly(D,L-

lysine), poly(diallyldimethylammonium chloride), poly(allylamine), poly(ethylene)imine,

chitosan, Poly(L-arginine), Poly(ornithine), Poly(D,L-hystidine), poly(mannoseamine,

and other sugars), combinations of polyamino acids and more generally any

combination of the polyamino acids and mixtures thereof.

46. (Currently Amended) The method according to claim 37, wherein the

polyelectrolyte multilayers can further comprise polymers with different functional

groups, including cationic (sulfonium, phosphonium, ammonium, hydroxylamine,

hydrazide), anionic (including poly(styrene sulfonate), poly(phosphate), polynucleic

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acid...) and neutral (including polyacrylamide, polyethylene oxyde, polyvinyl alcohol)

polymers.

47. (Currently Amended) The method according to claim 37, wherein the

polyelectrolyte multilayers comprise a variety of materials selected from , preferably

synthetic polyions (polymers presenting ions), biopolymers such as DNA, RNA,

collagen, peptides (such as a RGD sequence, Melanoma stimulating Hormone, or

buforin), proteins, [[and ]]enzymes, cells, viruses, dendrimers, colloids, inorganic

particles, [[and]] organic particles, dyes, vesicles, nano(or micro)capsules,

microcapsules, nano(or micro) particles, microparticles, polyelectrolytes complexes, free

<u>drugs</u>, [[or]] complexed drugs, cyclodextrins, and mixtures thereof.

48. (Previously Presented) The method according to claim 37, wherein the

coupling agent is a carbodiimide compound.

49. (Previously Presented) The method according to claim 37, wherein the

coupling agent is a compound of formula (I):

RN=C=NR'

wherein R and R', which are identical or different, represent an alkyl or aryl

group, preferentially an C1-C8 alkyl group.

50. (Previously Presented) The method according to claim 49, wherein the

coupling agent is 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC).

51. (Withdrawn) The method according to claim 37, wherein the coupling agent

is a peptide-coupling agent.

Claim 52. (Canceled)

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53. (Previously Presented) The method according to claim 37, wherein the

reaction of carboxylic groups and amino groups of the polyelectrolyte multilayers in the

presence of a coupling agent is carried out also in the presence of N-hydroxysulfo

succinimide para-nitrophenol, or dimethylaminopyridine.

54. (Withdrawn) A method of coating a surface, comprising (1) seguentially

depositing on a surface alternating layers of polyelectrolytes to provide a coated surface

presenting complementary reactive groups: amino and carboxylic groups, wherein a first

(or conversely second) polymer is a cationic polyelectrolyte and a second (or conversely

first) polymer is an anionic polyelectrolyte, and (2) reacting said complementary reactive

groups of the coated surface in the presence of a coupling agent, as to form amide

bonds between said complementary reactive groups, wherein step (2) is carried out also

in the presence of N-hydroxysuccinimide compounds.

55. (Withdrawn) The method according to claim 54, comprising (1) sequentially

bringing a surface into contact with polyelectrolyte solutions thereby adsorbing

alternated layers of polyelectrolytes to provide a coated surface presenting amino and

carboxylic groups, wherein a first (or conversely second) polymer is a cationic

polyelectrolyte and a second (or conversely first) polymer is an anionic polyelectrolyte,

and (2) reacting amino and carboxylic groups of the coated obtained surface in the

presence of a coupling agent, as to form amide bonds.

56. (Withdrawn) The method according to claim 54, wherein depositing on a

surface alternating layers of polyelectrolytes includes dipping, dip-coating, rinsing, dip-

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rinsing, spraying, inkjet printing, stamping, printing and microcontact printing, wiping, doctor blading or spin coating.

- 57. (Withdrawn) The method according to claim 54, wherein the depositing process involves coating and rinsing steps.
- 58. (Withdrawn) The method according to claim 54, wherein the carboxylic groups and amino groups are attached by covalent bonds to polyelectrolytes.
- 59. (Withdrawn) The method according to claim 54, wherein anionic polyelectrolytes which present free carboxylic groups are selected in the group consisting of polyacrylic acid, polymethacrylic acid, acid, poly(D,L-glutamic) acid, polyuronic acid (alginic, galacturonic, glucuronic...), glycosaminoglycans (hyaluronic acid dermatan sulphate, chondroitin sulphate, heparin, heparan sulphate, and keratan sulphate), poly(D,L-aspartic acid), any combination of the polyamino acids, and mixtures thereof.
- 60. (Withdrawn) The method according to claim 54, wherein cationic polyelectrolytes which present free amino groups are selected in the group consisting of poly(D,L-lysine), poly(diallyldimethylammonium chloride), poly(allylamine), poly(ethylene)imine, chitosan, Poly(L-arginine), Poly(ornithine), Poly(D,L-hystidine), poly(mannoseamine, and other sugars) and more generally any combination of the polyamino acids and mixtures thereof.
- 61. (Withdrawn) The method according to claim 54, wherein polyelectrolyte multilayers can further comprise polymers with different functional groups, including cationic (sulfonium, phosphonium, ammonium, hydroxylamine, hydrazide), anionic

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(including poly(styrene sulfonate), poly(phosphate), polynucleic acid...) and neutral (including polyacrylamide, polyethylene oxyde, polyvinyl alcohol) polymers.

- 62. (Withdrawn) The method according to claim 54, wherein the coupling agent is a carbodiimide compound.
- 63. (Withdrawn) The method according to claim 62, wherein the coupling agent is a compound of formula (I):

RN=C=NR'

wherein R and R', which are identical or different, represent an alkyl or aryl group, preferentially an C1-C8 alkyl group.

- 64. (Withdrawn) The method according to claim 54, wherein the coupling agent is 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC).
- 65. (Withdrawn) The method according to claim 54, wherein the coupling agent is a peptide coupling agent.

Claim 66. (Canceled)

- 67. (Withdrawn) The method according to claim 54, wherein step (2) is carried out also in the presence of N-hydroxysulfo succinimide para-nitrophenol, or dimethylaminopyridine.
- 68. (Withdrawn) The method according to claim 54, wherein the coated surface of step (1) further comprises a variety of materials, including synthetic polyions (polymers presenting ions), biopolymers such as DNA, RNA, collagen, peptides (such as a RGD sequence, Melanoma stimulating Hormone, or buforin), proteins, and enzymes, cells, viruses, dendrimers, colloids, inorganic or organic particles, dyes,

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vesicles, nano(micro)capsules and nano(micro)particles, polyelectrolytes complexes,

free or complexed drugs, cyclodextrins, and mixtures thereof.

69. (Withdrawn) A coated article obtained by a method according to claim 54.

70. (Withdrawn) A coated article obtained by a method according to claim 54,

wherein said coated article is biocompatible.

71. (Withdrawn) A coated article obtained by a method according to claim 54,

wherein said article is selected from the group consisting of blood vessel stents,

angioplasty balloons, vascular graft tubing, prosthetic blood vessels, vascular shunts,

heart valves, artificial heart components, pacemakers, pacemaker electrodes,

pacemaker leads, ventricular assist devices, contact lenses, intraocular lenses, sponges

for tissue engineering, foams for tissue engineering, matrices for tissue engineering,

scaffolds for tissue engineering, biomedical membranes, dialysis membranes, cell-

encapsulating membranes, drug delivery reservoirs, drug delivery matrices, drug

delivery pumps, catheters, tubing, cosmetic surgery prostheses, orthopedic prostheses,

dental prostheses, bone and dental implant, wound dressings, sutures, soft tissue repair

meshes, percutaneous devices, diagnostic biosensors, cellular arrays, cellular

networks, microfluidic devices, and protein arrays.

72. (Withdrawn) A coated article obtained by a method according to claim 54,

wherein said coated article further comprises a variety of materials, including synthetic

polyions, biopolymers such as DNA, RNA, collagen, peptides (such as a RGD

sequence, Melanoma stimulating Hormone, or buforin), proteins, and enzymes, cells,

viruses, dendrimers, colloids, inorganic and organic particles, vesicles,

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nano(micro)capsules and nano(micro)particles, dyes, vesicles, nano(micro)capsules and nano(micro)particles, polyelectrolytes complexes, free or complexed drugs, cyclodextrins, and mixtures thereof.

73. (new) The method according to claim 37, wherein the used polyelectrolyte multilayers are assembled via electrostatic attraction and hydrogen bridging.

74. (new) The method according to claim 44, wherein the polymers that constitute the multilayer film comprising anionic polyelectrolytes which present free carboxylic groups are selected from the group consisting of alginic acid, galacturonic acid, glucuronic acid, hyaluronic acid, dermatan sulphate, chondroitin sulphate, heparin, heparan sulphate, and keratan sulphate.

75. (new) The method according to claim 47, wherein the biopolymers are selected from DNA, RNA, collagen and peptides.

76. (new) The method according to claim 47, wherein the peptides are selected from a RGD sequence, Melanoma stimulating Hormone and buforin.